STUDIES ON THE HOLOCELLULOSE FRACTION OF HARDWOOD LEAVES[1]

B. B. COLDWELL[2] AND W. A. DELONG[3]

Department of Chemistry, Macdonald College, Que.

[Received for publication June 26, 1951]

INTRODUCTION

Theoretically, the holocellulose fraction of extractive-free plant materials comprises the entire polysaccharide content of the cell-wall. The preparation of holocellulose involves the removal of soluble and adsorbed substances of non-polysaccharide nature from plant tissues by extraction with suitable inert solvents, and delignification of the extractive-free residue. The first separation of such a fraction was made 30 years ago by Schmidt and Graumann (30), who quantitatively delignified wood with chlorine dioxide and sodium sulphite solution.

Various modifications of the original procedure have been proposed (32, 33, 31, 40, 41). The official method (tentative standard) now used for determining holocellulose in wood involves the alternate treatment of extractive-free sawdust with gaseous chlorine and alcoholic monoethanolamine solution (37).

A new technique based on delignification by acid sodium chlorite was developed by Jayme (16) in 1942 and has been simplified and improved by Wise and co-workers (43, 44). Unlike the earlier procedures (19), this method can be used for preparing large samples of holocellulose from woody and non-woody plants (5, 2, 34).

The objectives of the present work were: (*a*) to determine whether large samples of chlorite holocellulose could be prepared from fresh and partially decomposed leaves of different species; (*b*) to characterize the lignin-like material in the leaf tissue and that retained in the corresponding holocellulose fractions, and (*c*) to examine the hydrolytic products of the holocellulose fractions to obtain further information (12) respecting the nature of the decomposition processes. Research on the latter phase is still in progress.

MATERIALS AND METHODS

Mature, freshly-fallen, and partially decomposed leaves of the species, American beech (*Fagus grandifolia* Ehrh.), gray birch (*Betula populifolia* Marsh.), sugar maple (*Acer saccharum* Marsh.) and poplar (*Populus grandidentata* Michx. and *P. tremuloides* Michx.) were used. The sampling areas and the methods of collection and exposure of the leaves have been described previously (12).

Representative samples of the materials were air-dried in the laboratory and ground in a Wiley mill to pass a 20-mesh screen. Subsequent analyses were made on sub-samples of the ground material. Moisture was calculated as the loss in weight when two-gram samples were dried to constant weight in an electric oven at 105° C.; ash was found by igniting weighed samples in porcelain crucibles in a muffle at 600° C. Protein was calculated as nitrogen × 6.25, nitrogen being determined by the micro-Kjeldahl method (24). The preparation of extractive-free material has been described in a previous publication (12).

---

[1] Contribution from the Faculty of Agriculture, McGill University, Macdonald College, Que., and the Dominion Experimental Farms Service. Macdonald College Journal Series No. 288.

[2] Formerly Lecturer in Chemistry; now Chemist, Crime Detection Laboratory, R.C.M. Police, Ottawa, Canada.

[3] Associate Professor of Chemistry.

Lignin was determined by the 72 per cent sulphuric acid method (28) following pretreatment with 1 per cent hydrochloric acid (24).

Methoxyl was determined by the Vieböch and Schwappach modification of the Ziesel method as described by Clark (11) and accepted with slight changes as a tentative method by the A.O.A.C. (24). The air-dried samples were weighed out in gelatin capsules (size No. 1, Eli Lilly and Company) and a suspension of red phosphorus was used in the scrubber. These modifications were recommended by Samsel and McHard (29). Furfural was estimated photocolorimetrically by the Adams and Castagne (1) modification of the Stillings and Browning (36) method. Transmittance measurements were taken with a Coleman Universal Spectrophotometer model 11, employing a purple filter with the wavelength dial set at 5180 Å. Uronic acid anhydride was determined by the procedure of Dickson, Otterson and Link (13); alpha-cellulose by mercerization with 17.5 per cent sodium hydroxide as described by Ritter (27).

The method employed for the estimation of acetyl was essentially the Lemieux (18) semi-micro adaptation of the Genung and Mallatt (14) modified Ost distillation technique.

RESULTS AND DISCUSSION

Preparation of Leaf Holocellulose

Holocellulose was isolated according to the method of Jayme (16) as modified by Wise and co-workers (44). Two grams of air-dried, extractive-free leaves were weighed into a 400 ml. beaker, 100 ml. of distilled water were added and the beaker then placed in a water bath held at 75 ± 0.5° C, as recommended by Adams and Castagne (2). When the beaker contents reached the bath temperature, 0.12 ml. of glacial acetic acid, followed by 1.5 gm. of sodium chlorite (technical grade) were added. The beaker was covered with a watch glass and the contents stirred occasionally. At the end of one hour, without cooling, the same amounts of reagents were again added, the suspension thoroughly mixed and the heating continued. The treatment was repeated at the end of each hour until a theoretical yield of holocellulose was obtained (estimated by subtracting from 100 the sum of the ash-free lignin, protein, and ash contents of the extracted leaves). The suspension was filtered by suction through a sintered glass crucible (C porosity), washed repeatedly with water and finally with acetone. The holocellulose residue was dried at room temperature. The yield of holocellulose was calculated on a moisture-, lignin-, ash-, and protein-free basis.

A series of one to eight treatments were carried out on beech, birch, maple and poplar leaves, which were partially decomposed after 11 months' weathering in trays (12). The yields of holocellulose and the lignin, protein, and ash contents of the crude holocellulose fractions obtained on successive treatment of beech leaves with acid chlorite are shown in Figure 1. Similar results were obtained with birch, maple and poplar leaves.

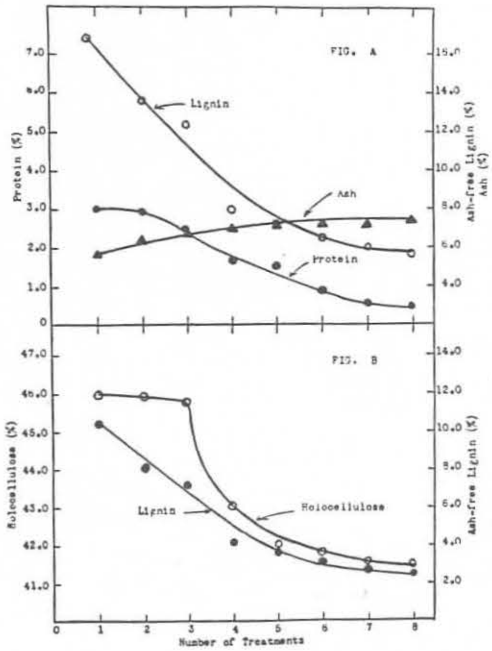

FIGURE 1. Effect of successive acid chlorite treatments on extractive-free beech leaves. (Exposed 11 months prior to analysis).

A. Ash, protein and ash-free lignin contents of crude holocellulose isolated after each treatment. (Basis: oven-dry crude holocellulose).

B. Yield of holocellulose and lignin content of the isolated fractions. (Basis: oven-dry extracted leaves).

Three treatments of beech, birch and maple leaves with acid chlorite gave theoretical yields of holocellulose. Poplar leaves required only two treatments (see Table 1). Longer digestion caused loss of holocellulose in all cases. The percentage of ash in the holocellulose fractions increased with each successive acid chlorite treatment. However, when calculated on the basis of extractive-free leaves, an actual decrease in ash occurred in all instances. Straw holocelluloses were found to contain more ash than the original extracted straw (2).

Delignification with sodium chlorite to obtain theoretical yields of holocellulose brought into solution from 74.4 to 84.0 per cent of the protein and from 77.2 to 87.1 per cent of the lignin on the basis of the oven-dry, extractive-free residues. The decrease in ash varied from 20.4 to 46.4 per cent. These results are shown in Table 1.

From the data given in Figure 1, it is evident that removal of lignin by successive acid chlorite treatments proceeded quite rapidly until the fourth treatment; the remaining lignin was tenaciously held by the cellulosic material and brought into solution only very slowly by further treatments. One chlorite treatment of beech leaves reduced the lignin from 38.54 to 10.51 per cent, based on the oven-dry extracted leaves. After four treatments the apparent lignin content was 4.14 per cent and four hours' further digestion decreased this only to 2.72 per cent. Complete removal of material reacting like lignin was not attained, even on prolonged treatment. Reference to Figure 1B shows that delignification treatments beyond three gave less than the theoretical yield of beech holocellulose and caused a progressive reduction in the carbohydrate material. Other workers report similar results in the preparation of holocellulose from cereal straws (2) and from wood (16, 44). The resistance of a part of the lignin to the action of

TABLE 1.—YIELDS OF HOLOCELLULOSE* AND REMOVAL OF LIGNIN, PROTEIN AND ASH ON DELIGNIFICATION OF EXTRACTED LEAVES WITH ACID CHLORITE

(Basis: 100 gm. oven-dry extracted leaves)

Species	Beech	Birch	Maple	Poplar
Number of chlorite treatments	3	3	3	2
Theoretical holocellulose content	45.5	27.4	43.4	35.5
Experimental yield of holocellulose	45.8	27.3	43.4	35.5
Percentage removal of lignin (ash-free)	81.2	87.1	77.2	83.4
Percentage removal of protein	74.4	80.1	84.0	76.6
Percentage removal of ash	26.0	46.4	20.4	42.1

* Corrected for content of ash, protein and ash-free lignin.

sodium chlorite may be due to the intimate association of lignin with cellulose; there is considerable evidence in support of a chemical union between lignin and carbohydrates in plant tissues (22, 26). Further, the effectiveness of sodium chlorite as an oxidizing agent in weak acid solutions decreases as the pH increases (38). In the experiments mentioned above, the pH of the suspensions increased in every case from about 4.2 to 5.0 during the first four treatments with chlorite, rising to 6.0 at the end of eight additions of the reagents. A similar increase in pH was noted by Adams and Castagne (2). Less effective delignification could be expected as the acidity decreased.

The results obtained on small samples indicated a fraction could be prepared containing most of the original carbohydrates in the plant tissue, freed from a large part of the lignin and protein. Such a fraction is particularly suited for research on the hemicelluloses which were of major interest. Therefore large lots of holocellulose (50 gm.) from mature leaves and residues were prepared by the method outlined for small samples, except that the suspension was stirred mechanically during the delignification step and the residual holocellulose was recovered and washed in a Buchner funnel lined with fine filter cloth. Proportionate amounts of reagents were added at hourly intervals and the temperature kept constant at 75 ± 0.5° C. Four chlorite treatments were employed, regardless of the species, in order to obtain fractions of low lignin content without prohibitive degradation of carbohydrate.

Analysis of Holocellulose from Freshly-Fallen and Partially Decomposed Leaves

Holocellulose fractions, prepared from freshly-fallen and from partially decomposed leaves by four successive treatments with acid chlorite, were analysed for ash, protein, ash-free lignin, furfural, uronic acid anhydride, acetyl and alpha-cellulose contents. The extracted leaves were analysed for the same constituents and the results are presented in Table 2. In order to facilitate comparison the data are presented on the basis of the moisture- and extractive-free leaf material.

It will be noted that four chlorite treatments resulted in appreciable losses of carbohydrates, except in fresh beech leaves. The loss of furfural from 1948 beech leaves was 2.9 per cent, while the decreases in uronic acid anhydride and acetyl contents were 20.1 and 25.3 per cent, respectively. In the other species of leaves collected in 1948, losses of these constituents ranged from 19.5 to 47.1 per cent. With the exception of birch leaves, the carbohydrate fraction in the partially decomposed material was more easily degraded than that present in the fresh leaves. Approximately 85 to 90 per cent of the protein and 80 to 85 per cent of the lignin in the extracted leaves were removed by four chlorite treatments. Removal

TABLE 2.—ANALYSIS OF LEAVES AND THEIR HOLOCELLULOSE FRACTIONS OBTAINED BY FOUR CHLORITE TREATMENTS

(Basis: Moisture- and extractive-free leaves and leaf residues)

Analyses (per cent)	Materials	Beech	Birch	Maple	Poplar
Unweathered leaves collected in 1947					
Experimental yield	Holocellulose	50.08	41.27	47.99	46.37
Theoretical yield	Holocellulose	49.79	46.72	52.81	50.86
Deviation of exptl. yield from theoretical	Holocellulose	+0.58	−11.66	−9.12	−8.82
Ash	Leaves	6.20	4.39	8.39	4.17
	Holocellulose	6.34	4.52	8.18	6.13
Protein (N × 6.25)	Leaves	5.49	8.56	6.92	5.83
	Holocellulose	0.74	0.90	1.00	0.72
Lignin, ash-free	Leaves	34.23	36.26	27.71	34.22
	Holocellulose	5.76	8.80	7.91	9.02
Alpha-cellulose	Holocellulose	—	—	32.31	32.37
1947 leaves exposed 11 months in trays					
Experimental yield	Holocellulose	42.78	25.53	37.50	29.38
Theoretical yield	Holocellulose	45.49	27.36	43.39	35.51
Deviation of exptl. yield from theoretical	Holocellulose	−5.95	−6.68	−13.57	−17.23
Ash	Leaves	5.30	7.11	12.05	6.44
	Holocellulose	4.49	4.57	9.57	4.46
Protein (N × 6.25)	Leaves	5.80	12.19	7.02	6.54
	Holocellulose	0.87	1.12	1.02	0.81
Lignin, ash-free	Leaves	38.54	49.91	33.04	46.87
	Holocellulose	6.41	6.81	6.63	7.96
Alpha-cellulose	Holocellulose	—	—	21.79	15.79
Unweathered leaves collected in 1948					
Experimental yield	Holocellulose	50.86	39.37	51.23	43.78
Theoretical yield	Holocellulose	51.12	42.25	53.89	48.12
Deviation of exptl. yield from theoretical	Holocellulose	−0.51	−6.81	−4.93	−9.01
Ash	Leaves	3.41	5.79	9.90	5.08
	Holocellulose	3.27	4.67	8.54	4.63
Protein (N × 6.25)	Leaves	5.92	11.12	5.48	4.85
	Holocellulose	0.78	0.88	0.52	0.42
Lignin, ash-free	Leaves	34.37	36.36	24.02	36.77
	Holocellulose	4.23	7.83	4.56	6.77
Furfural	Leaves	12.53	9.76	10.20	11.17
	Holocellulose	12.17	6.32	6.66	9.01
Uronic acid anhydride	Leaves	10.88	12.48	15.50	11.20
	Holocellulose	8.69	6.60	11.02	7.03
Acetyl	Leaves	3.98	2.05	2.64	2.84
	Holocellulose	2.97	1.46	1.84	1.98
Alpha-cellulose	Holocellulose	25.00	21.96	32.81	25.81

of ash was variable, though somewhat greater from the exposed leaves than from the unweathered leaves. The high ash contents of the holocelluloses prepared from 1947 beech, birch and poplar leaves are probably due to insufficient washing of these fractions with distilled water.

Characteristics of Leaf and Holocellulose Lignin

The nitrogen and methoxyl contents of lignin isolated from unweathered and partially decomposed leaves, and from the holocellulose fractions of these materials, are given in Table 3.

It will be noted that leaf lignin contains appreciable amounts of nitrogen. Lignin from freshly-fallen birch and maple leaves contains more nitrogen than that from beech and poplar. In general, from one-third to one-half of the nitrogen present in the leaf tissue is associated with the isolated lignin. Since treatment with chlorite is effective in removing a large portion of the nitrogen it is not surprising that holocellulose "lignin" contains relatively little nitrogen.

The methoxyl content of leaf lignin is low compared to that of lignin isolated from woods (15). The values are of the same magnitude as reported for lignin isolated from succulent plant tissues (20), soil (10), pasture herbage and feces of sheep (35). Beech leaf lignin has the highest methoxyl content, followed by maple, poplar and birch lignins. The data indicate that weathering has little effect on the composition of the leaf lignin.

TABLE 3.—THE NITROGEN AND METHOXYL CONTENTS OF THE KLASON LIGNIN ISOLATED FROM LEAVES, LEAF RESIDUES AND THEIR HOLOCELLULOSE FRACTIONS

(Basis: 100 gm. of moisture- and ash-free lignin)

Species	Lignin from leaves		"Lignin" from holocellulosee	
	N	OCH_3	N	OCH_3
Unweathered leaves collected in 1947				
Beech	1.32	6.75	0.44	2.28
Birch	1.96	3.81	0.29	1.13
Maple	2.08	5.20	0.51	3.80
Poplar	1.39	4.53	0.32	1.92
1947 leaves exposed in trays for 11 months				
Beech	1.55	7.16	0.31	2.64
Birch	2.06	4.25	0.33	1.02
Maple	1.65	6.78	0.40	2.07
Poplar	1.70	4.98	0.28	1.09
Unweathered leaves collected in 1948				
Beech	1.59	7.30	0.52	2.34
Birch	1.82	3.94	0.30	1.43
Maple	1.69	6.55	0.34	1.83
Poplar	1.14	4.91	0.18	0.48

"Lignin" isolated from holocellulose has a much reduced methoxyl content compared to that of lignin from the corresponding extractive-free tissue. Acid chlorite digestion either demethoxylates the lignin or removes methoxyl-containing interfering substances, or the material in holocellulose which is insoluble in 72 per cent sulphuric acid is quite different chemically from the acid-insoluble material in the original tissues. There is some basis for the latter suggestion. Holocellulose lignin was tough, elastic and nearly colourless compared to the dark chocolate brown, friable material isolated from the original tissue. So-called "lignin" isolated from leaf holocellulose may be carbohydrate in nature as it exhibits physical characteristics suggestive of plant gums or mucilages.

Determination of Sugars in Acid Hydrolysates of Leaf Holocelluloses

The holocellulose fractions prepared by four acid chlorite treatments of beech, birch, maple and poplar leaves (unweathered 1948 collection) were hydrolyzed with dilute sulphuric acid and the hydrolysates examined for sugars by the paper partition chromatographic technique.

In a typical experiment the holocellulose (0.5 gm.) was hydrolyzed by heating with 1 N sulphuric acid (12.5 ml.) in a sealed tube on a boiling water bath for 12 hours. The suspension was centrifuged and the insoluble residue washed with a small quantity of hot water. The filtrate and washings were combined and neutralized with solid barium carbonate using Congo red as the indicator. The barium sulphate was removed by centrifuging, washed with two to three millilitres of distilled water and the washings added to the filtrate which was concentrated to a syrup at room temperature. The water-soluble barium salts were precipitated by the addition of a large excess of ethanol and removed by centrifuging. The alcoholic filtrate was concentrated as above. The syrup was dissolved in two millilitres of distilled water and the solution examined for sugars as described in the following section.

The sugars were detected by descending paper chromatography as outlined by Partridge (25). The solvent system of ethyl acetate-acetic acid-water as recommended by Jermyn and Isherwood (17) was found suitable for the separation of xylose, glucose and galactose. Arabinose could not be positively identified with this solvent since mannose was found to have almost the same R_f value. The chromatograms were developed in the above solvent system for 24 and 36 hours. The longer period gave more complete separation of glucose and galactose. The presence of arabinose in the hydrolysates was ascertained by developing the chromatograms with phenol saturated with water. In this solvent arabinose moves faster than mannose and hence can be easily separated from it. The developed chromatograms were dried in an electric oven at 105° C. and the sugar spots made visible using the procedure outlined by Trevelyan, Procter and Harrison (39).

The effect of varying the concentration of sulphuric acid and the time of hydrolysis is well illustrated by the chromatograms shown in Figure 2. Hydrolysis of beech holocellulose with 0.2 N acid for one hour splitoff arabinose and small amounts of galactose and xylose. No glucose was present in the hydrolysate. Very faint spots of slow-moving substances are discernible; these are presumed to be di-, tri-, or higher polysaccharide fragments. Increasing the acid concentration to 1 N resulted in further hydrolysis of the holocellulose. Xylose, arabinose and galactose show well defined spots, the spots presumed due to polysaccharide fragments are more pronounced and the presence of glucose in the hydrolysate is indicated. On lengthening the time of hydrolysis to four hours the spots indicating incompletely hydrolysed fragments disappeared. Beech holocellulose appears to be completely hydrolysed in four hours as no more sugars were detected when the hydrolysis was continued for 12 hours. On the basis of these results all further hydrolyses were carried on for 12 hours using 1 N sulphuric acid.

Xylose, arabinose, glucose and galactose were detected in each of the holocellulose fractions examined. Figure 3 is a photograph of the original chromatograms of maple hydrolysate, developed as described above. A mixture of known sugars are spotted on each chromatogram for purposes of comparison. Similar results were obtained with hydrolysates of beech, birch and poplar holocellulose.

Xylose, arabinose and galactose appear to be relatively common constituents of plant hemicelluloses (3, 4, 6, 7, 8, 9, 21, 23, 42), whereas glucose is seldom encountered. Recently glucose was specifically identified as a constituent of wheat straw hemicellulose (6). In the present study, glucose may have resulted from hydrolysis of alpha-cellulose although this would not be expected to occur to any extent in a dilute acid solution. More detailed investigation of the holocellulose fractions is desirable.

SUMMARY

Chlorite holocellulose fractions of extractive-free hardwood leaves apparently retained 13 to 23 per cent of the original lignin and from 16 to 26 per cent of the original protein present in the tissues.

Beech, birch and maple leaves required three treatments and poplar leaves two treatments with acid chlorite to obtain a theoretical yield of holocellulose.

A relatively large proportion of the lignin in extracted leaves is easily brought into solution by the reagent while a small amount appears to be tenaciously held and not removed by chlorite without simultaneous degradation of the carbohydrate substances.

With the exception of that from birch, the carbohydrate fractions in partially decomposed leaves were more readily attacked by chlorite than those present in unweathered leaves.

Hardwood leaf lignin contains appreciable amounts of nitrogen; from one-third to one-half of the nitrogen present in the leaf tissue is associated with the isolated lignin.

The methoxyl contents of the various leaf lignins ranged from 3.81 to 7.30 per cent, being highest in beech followed by maple, poplar and birch. Lignin isolated from partially decomposed leaves (1947 collection) contained more methoxyl than lignin from the corresponding unweathered leaves.

Lignin isolated from holocellulose had a much reduced nitrogen and methoxyl content, compared to that of lignin from the corresponding extractive-free tissue.

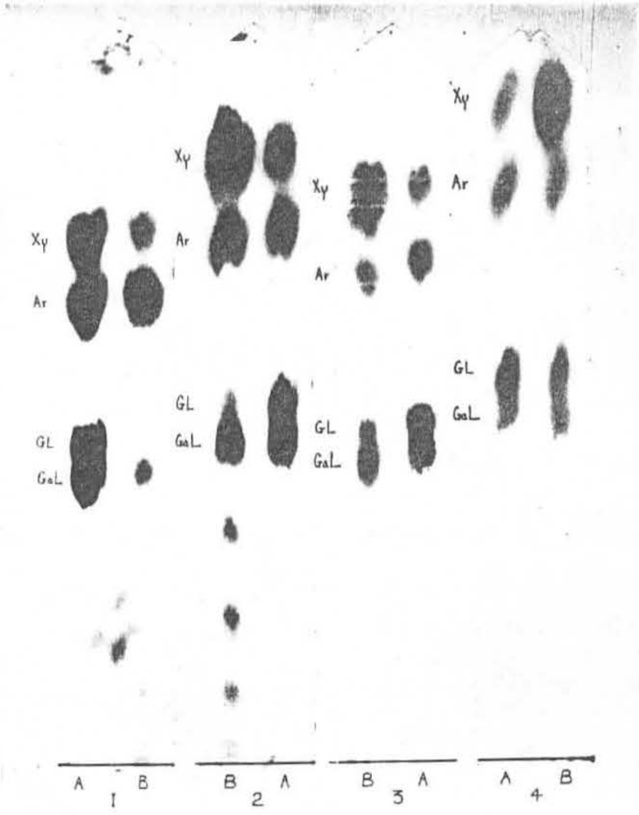

FIGURE 2. Hydrolysis of beech holocellulose for different periods.
A = known sugars; B = hydrolysate; X_y = xylose;
Ar = arabinose; Gl = glucose; Gal = galactose.
No. 1—0.2 NH_2SO_4; time—1 hr.
No. 2—1.0 NH_2SO_4; time—1 hr.
No. 3—1 NH_2SO_4; time—4 hr.
No. 4—1 NH_2SO_4; time—12 hr.

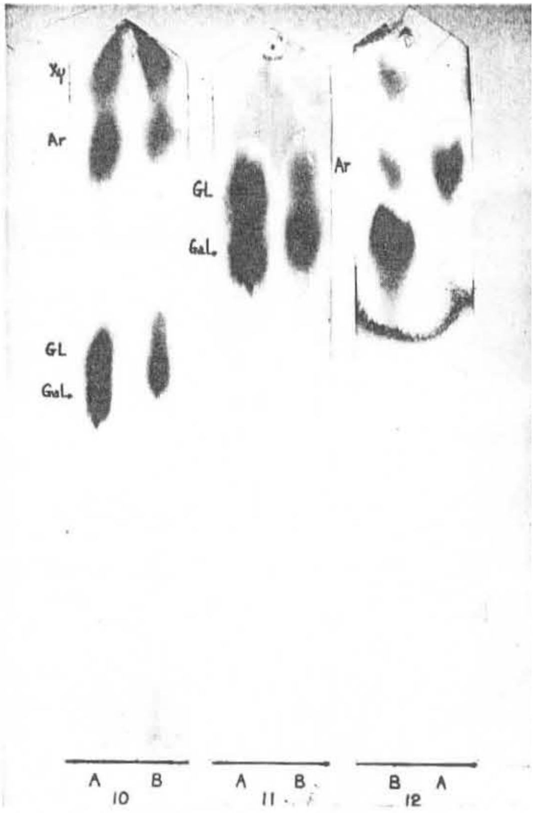

FIGURE 3. Chromatograms of hydrolysate from maple holocellulose.
A = known sugars; B = hydrolysate;
Xy = xylose; Ar = arabinose;
Gl = Glucose; Gal = galactose.
No. 10—Time of run 24 hr.; solvent—EtAc + HAc + H_2O
No. 11—Time of run 36 hr.; solvent—EtAc + HAc + H_2O
No. 12—Time of run 36 hr.; solvent—Phenol.

Lignin isolated from leaf holocellulose was tough, elastic, and nearly colourless, compared to the dark chocolate brown, friable material obtained from the original tissue, which suggests that the two lignins differ in chemical composition.

Xylose, arabinose, glucose and galactose were identified by paper chromatography, in the acid hydrolysates of beech, birch, maple and poplar leaf holocellulose.

ACKNOWLEDGMENTS

The authors acknowledge with gratitude their indebtedness to officers of the Experimental Farms Service, Canada Department of Agriculture, whose advice, support and assistance made this investigation possible.

REFERENCES

1. Adams, G. A., and A. E. Castagne. Some factors affecting the determination of furfural. Can. J. Research, *B*, 26 : 314-324. 1948.
2. Adams, G. A., and A. E. Castagne. Holocelluloses from straws. Canadian J. Research, *B*, 26 : 325-334. 1948.
3. Anderson, E., and P. W. Krznarich. Hemicellulose from oat hulls. J. Biol. Chem. 111 : 549-552. 1935.
4. Anderson, E., and H. J. Lowe. The composition of flaxseed mucilage. J. Biol. Chem. 168 : 289-297. 1947.
5. Bennett, E. Preparation of holocellulose from non-woody plant material. Anal. Chem. 19 : 215. 1947.
6. Bishop, C. T., and G. A. Adams. Use of liquid ammonia in the fractionation of wheat straw holocellulose. Can. J. Research, *B*, 28 : 753-761. 1950.
7. Buston, H. W. The polyuronide constituents of forage grasses. Biochem. J. 28 : 1028-1037. 1934.
8. Buston, H. W. Observations on the nature, distribution and development of certain cell-wall constituents of plants. Biochem. J. 29 : 196-218. 1935.
9. Buston, H. W., and V. H. Chambers. Some cell-wall constituents of *Cetraria islandica* ("Iceland Moss"). Biochem. J. 27 : 1691-1701. 1933.
10. Cann, E. D. Fractionation of soil organic matter. *Doctoral Dissertation*, McGill University. 1946.
11. Clark, E. P. The Vieböck and Schwappach method for the determination of methoxyl and ethoxyl groups. J. Assoc. Official Agr. Chem. 15 : 136-140. 1932.
12. Coldwell, B. B., and W. A. DeLong. Studies of the composition of deciduous forest tree leaves before and after partial decomposition. Sci. Agr. 30 : 456-466. 1950.
13. Dickson, A. D., H. Otterson, and K. P. Link. A method for the determination of uronic acids. J. Amer. Chem. Soc. 52 : 775-779. 1930.
14. Genung, L. B., and R. C. Mallatt. Analysis of cellulose derivatives. Ind. Eng. Chem., *Anal Ed.* 13 : 369-374. 1941.
15. Harris, E. E. Some characteristics of wood lignins. J. Amer. Chem. Soc. 58 : 894-897. 1936.
16. Jayme, G. Preparation of holocellulose and cellulose with sodium chlorite. Cellulosechemie, 20 : 43-49. 1942. *Abstracted in* Chem. Abstracts 37 : 766. 1943.
17. Jermyn, M. A., and F. A. Isherwood. Improved separation of sugars on the paper partition chromatogram. Biochem. J. 44 : 402-407. 1949.
18. Lemieux, R. U. "Hindrance effects" in cellulose substitution reactions. *Doctoral Dissertation*, McGill University. 1946.
19. Lovell, E. L. Fibrous holocellulose from softwoods. Ind. Eng. Chem. 37 : 1034-1037. 1945.
20. MacDougall, D., and W. A. DeLong. Effect of initial drying temperature on the apparent lignin content of plant tissues. Can. J. Research, *B*, 20 : 40-48. 1942.
21. McIlroy, R. T., G. S. Holmes, and R. P. Mauger. A preliminary study of the polyuronide hemicellulose of *Phormium tenax* (N.Z. flax). J. Chem. Soc. (London), pp. 796-799, 1945.
22. Norman, A. G. *In* Cellulose and cellulose derivatives, pp. 427-446, ed. by Emil Ott. Interscience Publishers, Inc., New York, N.Y. 1943.
23. Norris, F. W., and I. A. Preece. Studies on hemicelluloses. I. The hemicelluloses of wheat bran. Biochem. J. 24 : 59-66. 1930.

REFERENCES—Continued

24. Official and tentative methods of analysis, 6th ed. Assoc. of Official Agr. Chemists. Washington, D.C. 1945.
25. Partridge, S. M. Application of the paper partition chromatogram to the qualitative analysis of reducing sugars. Nature 158 : 270-271. 1946.
26. Purves, C. B. *In* Cellulose and cellulose derivatives, pp. 101-106, ed. by Emil Ott. Interscience Publishers, Inc., New York, N.Y. 1943.
27. Ritter, G. J. Determination of alpha-cellulose. Ind. Eng. Chem., *Anal. Ed.* 1 : 52-54. 1929.
28. Ritter, G. R., R. M. Seborg, and R. L. Mitchell. Factors affecting quantitative determination of lignin by 72 per cent sulfuric acid method. Ind. Eng. Chem., *Anal. Ed.* 4 : 202-204. 1932.
29. Samsel, E. P., and J. A. McHard. Determination of alkoxyl groups in cellulose ethers. Ind. Eng. Chem., *Anal. Ed.* 14 : 750-754. 1942.
30. Schmidt, E., and E. Graumann. Incrusting substances of plants. I. Methods for the preparation of pure plant skeletal substances. Ber. B, 54 : 1860-1873. 1921. *Abstracted in* Chem. Abstracts 16 : 273. 1922.
31. Schmidt, E., K. Meinel, W. Jandebeur, and W. Simson. Die Kettelänge der Cellulosen Nativer Zusammensetzung und die Kettenlänge des Acetyl-Xylans der Laubhölzer. Cellulosechemie 13 : 129-139. 1932.
32. Schmidt, E., K. Meinel, and E. Zinth. Plant membranes. Ber., B, 60 : 503-526. 1927. *Abstracted in* Chem. Abstracts 21 : 1829. 1927.
33. Schmidt, E., Y. Tang, and W. Jandebeur. Preparation of skeleton substances from encrusted cell-walls by chlorine dioxide in the "one step procedure". Cellulosechemie 12 : 201-212. 1931. *Abstracted in* Chem. Abstracts 26 : 1772. 1932.
34. Sohn, A. W., and F. Reiff. Sodium chlorite as a pulping agent. Papier-Fabr. 40 : 1-7 : 105-112. 1942. *Abstracted in* Chem. Abstracts 37 : 1865. 1943.
35. Sowden, J. F. Studies on lignin and related compounds in forage and in animal excreta. *Doctoral Dissertation*, McGill University. 1944.
36. Stillings, R. A., and B. L. Browning. Photocolorimetric determination of furfural. Ind. Eng. Chem., *Anal. Ed.* 12 : 499-502. 1940.
37. TAPPI official standard T 9 M-45. Sept., 1945.
38. Taylor, M. C., J. F. White, G. P. Vincent, and G. L. Cunningham. Sodium chlorite. Ind. Eng. Chem. 32 : 899-903. 1940.
39. Trevelyan, W. E., D. P. Procter, and J. S. Harrison. Detection of sugars on paper chromatograms. Nature 166 : 444-445. 1950.
40. Van Beckum, W. G., and G. J. Ritter. Rapid methods for the determination of holocellulose and Cross and Bevan cellulose in wood. Paper Trade J. 105 : No. 18, pp. 127-130. 1937.
41. Van Beckum, W. G., and G. J. Ritter. Chemical composition of wood. Paper Trade J. 104 : No. 19, pp. 49-50. 1937.
42. Weihe, H. D., and M. Phillips. Hemicelluloses of wheat straw. J. Agr. Research 60 : 781-786. 1940.
43. Wise, L. E. Quantitative isolation of hemicelluloses from coniferous woods. Ind. Eng. Chem., *Anal. Ed.* 17 : 63-64. 1945.
44. Wise, L. E., M. Murphy, and A. A. D'Addieco. Chlorite holocellulose, its fractionation and bearing on summative wood analysis and on studies on the hemicelluloses. Paper Trade J. 122 : No. 2, pp. 35-43. 1946.